Research Article

Biodiesel synthesis from palm fatty acid distillate using enzyme immobilized on magnetic nanoparticles

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Abstract
The present study deals with the esterification of palm fatty acid distillate (PFAD) using immobilized lipase on magnetic nanoparticles (MNPs) to produce biodiesel in a cleaner and more environmentally friendly way. Commercially available lipase $CALB_{EX}$ was used for immobilization of lipase on MNPs. The effect of various reaction parameters including the methanol-to-PFAD molar ratio, biocatalyst loading, reaction temperature, and agitation speed was examined using a one-factor-at-a-time approach. Maximum PFAD conversion of 82.74% was achieved under mild reaction conditions, with a methanol-to-PFAD molar ratio of 1.6:1, biocatalyst loading of 8 wt% (lipase content 0.45 wt%), agitation speed of 150 rpm, reaction temperature of 50 °C, and reaction time of 10 h. The catalytic activity of MNP-$CALB_{EX}$ was compared with commercial Amberlyst-15 under similar reaction conditions. It was observed that MNP-$CALB_{EX}$ achieved 2.6-fold higher conversion than Amberlyst-15. The reusability of the immobilized biocatalyst was also tested to determine cost feasibility. It was observed that the immobilized biocatalyst could be used up to five cycles, with residual conversion of 80.19% in the fifth cycle.

Keywords FAME · PFAD · Enzyme · Immobilization · Magnetic nanoparticles

1 Introduction

Energy consumption has increased globally due to population explosion and industrialization, resulting in a serious energy crisis [1]. In the current scenario, the energy required in the transport sector is supplied by conventional fossil fuels like petroleum and natural gas [2]. Fossil fuels are non-renewable energy sources, and are the primary source of greenhouse gas emissions in the environment [3]. Also, the continuous hike in the price of the limited stock of fossil fuel has caused policymakers to consider alternative options such as biodiesel and bioethanol, especially in developing countries like India [4]. India ranked third in the world after the USA and China in oil consumption in 2018 [5]. Moreover, there is a huge gap between domestic crude oil production and consumption. India's crude oil production has declined continuously over the past 7 years (2012–2018), with dependence on imports rising to 84% [6]. India spent Rs 4.7 lakh crore, or USD 70.196 billion, on crude oil imports in 2016–2017 [7]. However, biofuel can be used to replace a percentage of liquid fossil fuel, which can help in reducing the massive outflow of foreign currency [8]. In this context, the Ministry of Petroleum and Natural Gas, Government of India, approved a national policy on biofuel in 2018, targeting a 5% blend of biodiesel in diesel and 20% blend in ethanol in petrol by 2030 [9].

Biodiesel or fatty acid methyl ester (FAME) is a renewable and biodegradable fuel alternative to petroleum diesel. It can be produced from the transesterification of edible oils such as sunflower, palm, soybean, or corn oil, from esterification followed by transesterification of non-edible

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oils such as *Jatropha curcas*, *Pongamia pinnata* (karanja oil), linseed, *Azadirachta indica* (neem oil), or *Ricinus communis* (castor oil), or by esterification of palm fatty acid distillate [10] with short-chain alcohols (methanol or ethanol) [11]. The production of biodiesel from edible oil is not economically viable, as it not only increases production costs but also competes with food production, leading to major nutritional and ethical concerns [12]. Therefore, cheap, non-edible, and readily available feedstocks can solve the problems associated with edible oil. Palm fatty acid distillate (PFAD) is a cheap and readily available feedstock (INR 10–30/kg) for biodiesel production, as it is formed as a residue during the palm oil refining process [13]. PFAD contains about 93% free fatty acids (FFA), and requires acid catalysts to carry out the esterification reaction [14].

Sulfuric acid is commonly used as a catalyst for the transformation of PFAD to FAME. However, due to the corrosive nature of sulfuric acid, costly equipment is required for handling, which results in higher production costs. Additionally, after completion of the reaction, a neutralization process must be carried out, which generates a large quantity of wastewater. Therefore, many studies in the literature have reported the use of a heterogeneous acid catalyst (CM-SO_3H, sulfonated graphene, sulfated LaO) as a suitable candidate to promote the esterification reaction [15–17]. Though heterogeneous acid catalysts are a better alternative to homogeneous acid catalysts, the reaction conditions are comparatively harsh, requiring high temperature and a large amount of methanol (MeOH). Hence, research scientists have investigated the use of lipase as biocatalyst, which can be performed under very mild reaction conditions [18].

Furthermore, lipase can esterify/transesterify a wide variety of edible and non-edible feedstocks, irrespective of the presence of FFA and some amount of water, and also produces a pure and high-quality product (biodiesel), with glycerol as by-product [19]. Lipase-catalyzed biodiesel production is a greener, cleaner, and safer process. However, the use of lipase in free form is not cost-effective. Consequently, different techniques have been developed to immobilize lipase so that it can be recycled to reduce biodiesel production costs. Babaki et al. [20] immobilized lipase on silica nanoparticles for the transesterification of canola oil.

Similarly, Macario et al. [21] synthesized lipase-encapsulated silica nanoparticles for the transesterification of triolein to produce biodiesel. In another study, Miao et al. [22] prepared a biocatalyst by immobilization of lipase on amino-functionalized magnetic nanoparticles (MNPs). The prepared catalyst was tested for FAME synthesis from rapeseed oil. The authors reported maximum conversion of 89.4% under optimal reaction conditions, i.e., molar ratio of 6:1, catalyst loading of 20 wt%, water content of 2%, temperature of 45 °C, and agitation speed of 250 rpm over a reaction period of 24 h. Xie and Wang [23] also immobilized lipase on magnetic Fe_3O_4/poly(styrene-methacrylic acid) microspheres for the transesterification of soybean oil.

However, a detailed review of the literature revealed no published work on the esterification of PFAD using lipase immobilized on Fe_3O_4-NH_2 magnetic nanoparticles. Therefore, the present work is focused on the detailed study of esterification of PFAD in the presence of a biocatalyst in the form of immobilized lipase on Fe_3O_4 magnetic nanoparticles for biodiesel synthesis. The immobilized biocatalyst was characterized by Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy TEM, and Brunauer–Emmett–Teller (BET) analysis. The influence of various reaction conditions including PFAD-to-methanol molar ratio, biocatalyst loading, reaction temperature, and agitation speed was also examined.

2 Materials and methods

2.1 Materials

PFAD was procured from Godrej Industries Ltd. (Mumbai, India). The fatty acid composition and properties of the PFAD are presented in Table 1, and are similar to our previous work [1]. Ferrous sulfate ($FeSO_4.7H_2O$), ferric chloride (FeCl3), 25% ammonia solution, ethanol, methanol (MeOH), glutaraldehyde, potassium hydroxide (KOH), isopropanol, gum arabic, p-nitrophenyl acetate (pNPA), chloroform, and isoamyl alcohol were all AR grade and purchased from SD Fine-Chem Ltd., Mumbai. The free

Table 1 Fatty acid composition and properties of PFAD

PFAD	Value
Fatty acid composition	
Palmitic acid (%)	49.6
Stearic acid (%)	4.7
Oleic acid (%)	35.3
Linoleic acid (%)	9.1
Others (%)	1.3
Properties	
Saponification value (mg of KOH/g of PFAD)	198.2
Acid value (mg of KOH/g of PFAD – AOCS Te 2a-64)	186.1
Viscosity (mm^2/s)	38.4
Density (g/cm^3)	0.919
Water content (%)	0.14
Molecular weight (g/mol)	267.9

enzyme *Candida antarctica* lipase B ($CALB_{EX}$ 10,000) was kindly donated by Fermenta Biotech Ltd. (Thane, India). The methyl esters including methyl palmitate, methyl oleate, methyl stearate, and methyl linoleate of HPLC grade were procured from Sigma-Aldrich.

2.2 Methods

2.2.1 Lipase activity assay

Lipase activity was measured spectrophotometrically by hydrolysis of p-nitrophenyl acetate (pNPA) to p-nitrophenol (pNP) at 405 nm using a UV–Vis spectrophotometer (Jasco, USA) [24]. Briefly, 0.1 mL of 0.1 M pNPA (prepared in isopropanol) was added to 1.9 mL of buffer solution (0.1 M sodium phosphate pH 7.0) and heated to 40 °C. When the temperature reached to 40 °C, lipase was added and the mixture was incubated for 5 min. After incubation, 2 mL of Marmur solution (chloroform:Isoamyl alcohol 24:1) was added to stop the reaction. Centrifugation of the mixture was then carried out at 8000 rpm for 3 min at 4 °C. The bright yellowish solution (p-nitrophenol) from the top was extracted to determine the enzyme activity. One unit (U) of enzyme activity is defined as µmol of pNPA transformed into pNP per min during hydrolysis at optimal conditions. Lipase activity was found to be 8.56 U mg^{-1}.

2.2.2 Catalyst preparation

The catalyst was synthesized in two steps, as previously reported [25]. Firstly, $FeSO_4.7H_2O$ and $FeCl_3$ in a 2:1 molar ratio were added to distilled water at room temperature (34 °C) under continuous nitrogen gas purging to avoid probable oxidation. Once the $FeSO_4.7H_2O$ and $FeCl_3$ mixture was completely dissolved, aqueous ammonia solution was added dropwise under vigorous stirring until the pH of the solution reached to 10. At this pH, Fe_3O_4 was precipitated out and separated using a magnet. The isolated precipitate was lyophilized for 48 h under vacuum to obtain amine-functionalized Fe_3O_4 magnetic nanoparticles.

In the second step, the immobilization of $CALB_{EX}$ 10,000 on amine-functionalized Fe_3O_4 magnetic nanoparticles was performed. Firstly, 750 mg of Fe_3O_4-NH_2 nanoparticles was dispersed in 5 mL of deionized water by sonication. The dispersed Fe_3O_4-NH_2 nanoparticles were mixed with 5 mL $CALB_{EX}$ 10,000 (10 mg/mL in phosphate buffer solution), and then 1 mL of 25% glutaraldehyde solution was added to the mixture. This mixture was incubated at 30 °C in an orbital shaker at 150 rpm for 50 min. After incubation, immobilized magnetic nanoparticles were separated from the supernatant by a magnet and washed five to six times with distilled water and air-dried. The synthesized nanocatalyst was labeled MNP-$CALB_{EX}$. The percent activity recovery of lipase in immobilized form was determined from Eq. 1.

$$\text{Activity recovery}\ (\%) = \frac{\text{The activity of immobilized lipase}}{\text{The activity of free lipase}} \times 100 \quad (1)$$

2.2.3 Immobilization efficiency

Immobilization efficiency is defined as the ratio of the amount of enzyme ($CALB_{EX}$ 10,000) immobilized to the total amount of the enzyme used [26], as given in Eq. 2. The amount of enzyme immobilized is the difference between the initial enzyme concentration and the non-immobilized enzyme concentration found in the supernatant. The protein concentration in the supernatant was evaluated by the Bradford assay, as described elsewhere [27].

$$\text{Immobilization efficiency}\ (\%) = \frac{\text{Amount of enzyme immobilized to } Fe_3O_4{-}NH_2}{\text{Amount of enzyme used}} \times 100 \quad (2)$$

2.2.4 Catalyst characterization

The Fourier transform infrared (FT-IR) spectra of MNP-$CALB_{EX}$, $CALB_{EX}$, and MNP-NH_2 were recorded on an IRAffinity-1 FTIR spectrometer. TEM images of MNP-$CALB_{EX}$ were acquired using a JEOL JEM-2100 instrument. The specific surface area of MNP-$CALB_{EX}$ was obtained using a BET sorptometer (Porous Materials Inc., USA).

2.2.5 Reaction procedure for the esterification of PFAD using MNP-$CALB_{EX}$

The esterification reaction of PFAD was performed in a 20 mL baffled glass reactor with a three-neck lid fitted with a stirrer, reflux glass condenser, and thermometer. Firstly, a known amount of PFAD was added to the glass reactor and heated at 45 °C with the help of a thermostatic water bath to convert solid PFAD into liquid. After liquefaction, a pre-calculated quantity of MeOH and immobilized lipase (MNP-$CALB_{EX}$) was added to start the reaction. Aliquots from the esterified reaction mixture were taken off at a fixed interval of time and analyzed with standard acid value and gas chromatography (GC) methods. The

effects of different operational parameters including the MeOH-to-PFAD molar ratio, catalyst dosage, reaction temperature, and agitation speed were examined using a one-factor-at-a-time approach [28]. All the experiments were carried out in duplicate, and the average values are shown in the figures.

2.2.6 Analysis

Sample titration was carried out using an alcoholic potassium hydroxide solution to determine the amount of unconverted PFAD in terms of its acid value [14]. Instrumental analysis of the optimized sample was performed using a gas chromatograph (Clarus 580, PerkinElmer) equipped with a flame ionization detector along with a BPX70 capillary column (25 m × 0.25 mm with 0.25 µm film thickness). The sample was diluted in hexane and injected with 0.2 µL volume. Nitrogen gas was used as a carrier gas. The injector and detector temperatures were set at 220 °C and 240 °C, respectively. The oven temperature was programmed in the following steps: 70 °C for 2 min; 10 °C/min to 190 °C; then 5 °C/min to 240 °C and held for 10 min. The percentage conversion of PFAD was determined by Eq. 3.

$$\text{Conversion}(\%) = \frac{AV_i - AV_t}{AV_i} \times 100, \quad (3)$$

where AV_i is the initial acid value (without the addition of MNP-$CALB_{EX}$) and AV_t is the acid value at time *t* (with the addition of MNP-$CALB_{EX}$) of the esterified reaction mixture.

2.2.7 Reusability of the biocatalyst

The reusability of the magnetic biocatalyst was evaluated for the esterification of PFAD with MeOH in a batch reactor at the optimized reaction parameters. After completion of the esterification reaction, immobilized lipase was separated out using a magnet and washed with *n*-hexane. The washed magnetic biocatalyst was reused in the next cycle. The conversion of the first cycle was set as 100%, and residual conversion in subsequent cycles was estimated accordingly.

3 Results and discussion

3.1 Characterization of MNP-$CALB_{EX}$

The MNP-$CALB_{EX}$ biocatalyst was characterized by TEM, BET, and FTIR, while activity was assayed using hydrolysis of pNPA, and immobilization efficiency was evaluated by the Bradford assay.

The FTIR spectra of MNP-NH_2, MNP-$CALB_{EX}$, and $CALB_{EX}$ are shown in Fig. 1a–c. The absorption band at 581 cm^{-1} and 575 cm^{-1} is a characteristic peak of F_3O_4 MNPs, which indicates the stretching vibration of Fe–O [29]. For MNP-NH_2 (Fig. 1a), the peaks observed at 3170 cm^{-1} and 3113 cm^{-1}, and 1686 cm^{-1} are mainly attributed to stretching and bending vibrations of the primary amine (–NH_2), respectively [22]. In Fig. 1b, the absorption peaks at 1640 cm^{-1} and 1036 cm^{-1} correspond to the characteristic peaks of pure lipase ($CALB_{EX}$) [30] which were observed at 1630 cm^{-1} and 1075 cm^{-1} in the FTIR spectrum of immobilized lipase (MNP-$CALB_{EX}$) (Fig. 1c). Broad absorption peaks of the hydroxyl group appear at 3392 cm^{-1} and 3321 cm^{-1} (Fig. 1b–c) due to stretching vibrations.

TEM analysis was carried out to determine the particle size and morphology of MNP-$CALB_{EX}$. The magnified TEM images of magnetic nanoparticles are shown in Fig. 2. The TEM image at magnification of ×200,000 revealed that the magnetic Fe_3O_4 nanoparticles were almost spherical or ellipsoid, with particle size of 10–20 nm.

BET analysis of Fe_3O_4 MNPs was carried out to determine the specific surface area and pore volume, which revealed values of 143.26 m^2/g and 0.18 cm^3/g, respectively. A large surface area aids in the anchoring of a large amount of lipase on its surface. The lipase loading found in the present work was 56 mg/g of support, which is much higher than that for enzyme immobilized on a silica nanoparticle support (36 mg/g support) reported in the literature [20]. The lipase immobilization efficiency was calculated from Eq. 2 and was found to be 85%.

3.2 Effect of biocatalyst loading

Industrial-scale enzymatic biodiesel production cost is largely affected by the higher cost of the enzyme. Also, enzyme loading has a significant impact on the conversion of PFAD to biodiesel. Therefore, it is necessary to optimize the amount of catalyst loading to reduce production cost while achieving maximum conversion. Hence, the effect of catalyst (MNP-$CALB_{EX}$) loading on the percentage PFAD conversion was studied in the range of 4–10 wt% while other parameters including temperature, molar ratio, and agitation speed were kept constant, and the results obtained are depicted in Fig. 3. It can be observed that the conversion of PFAD increased from 54.46% at 4 wt% dose of MNP-$CALB_{EX}$ to 82.74% at 8 wt% of MNP-$CALB_{EX}$ (lipase content 0.45 wt%). As the reaction occurred at the interface of the biocatalyst and reactants, increasing the biocatalyst dose increased the interfacial area for the interaction between catalyst and reactants to form an enzyme–substrate complex that was then transformed into the product

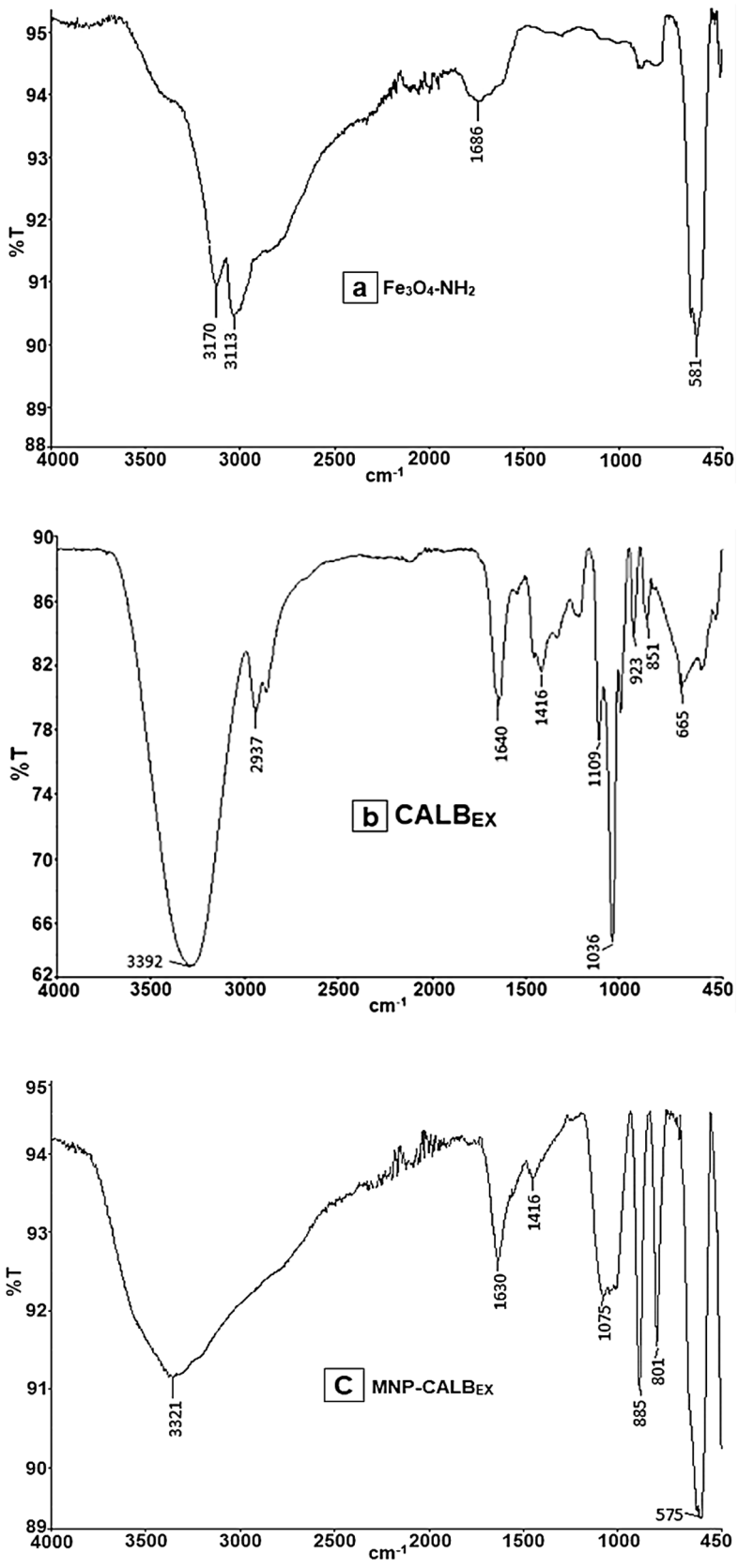

Fig. 1 FT-IR spectra of MNP-NH_2 (**a**), free lipase ($CALB_{EX}$) (**b**), and MNP-$CALB_{EX}$ (**c**)

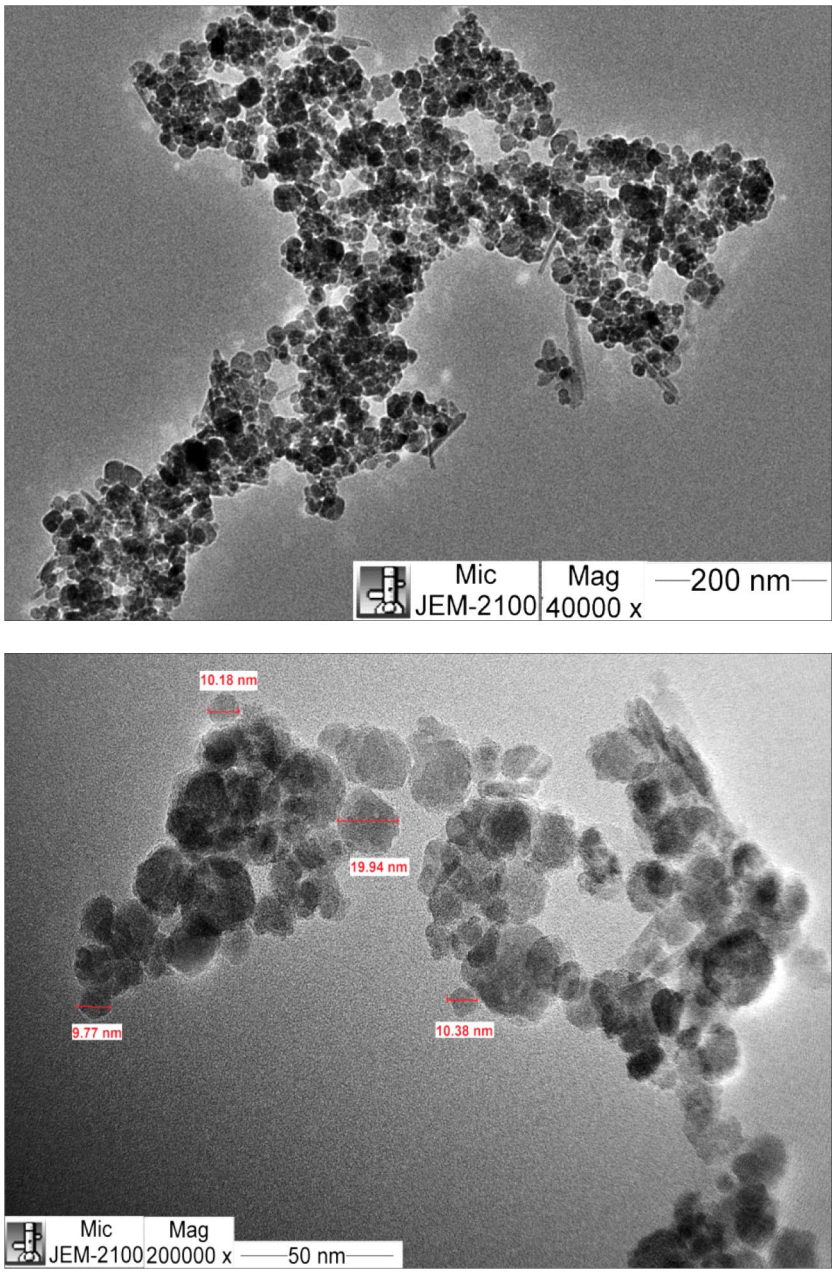

Fig. 2 TEM images of MNP-$CALB_{EX}$ at ×40,000 and ×200,000 magnification

and enzyme. Gharat and Rathod [31] also reported that higher biocatalyst loading enhanced the formation of the enzyme–substrate complex due to an increase in the active catalytic sites, and thus the conversion increased.

However, a further increase in catalyst loading (10 wt%) showed an insignificant increase in conversion. This may be due to the saturation of the interface with enzyme, and hence a further increase in enzyme loading showed no enhancement in conversion. Hence, 8 wt% was considered as optimum MNP-$CALB_{EX}$ loading. Adewale et al. [32] investigated the effect of enzyme concentration on the production of biodiesel from waste tallow. They reported that an increase in enzyme concentration from 4 wt% to 6 wt% resulted in an increase in biodiesel yield from 60% to 85.6%, while a further increase in enzyme concentration (8 wt%) showed no difference in yield. Similarly, Pedro et al. [33] studied the influence of enzyme (Novozym 435) loading on ester content by varying the amount of catalyst from 1 wt% to 9 wt%. The highest ester content of 73% was reported at enzyme loading of 9 wt%.

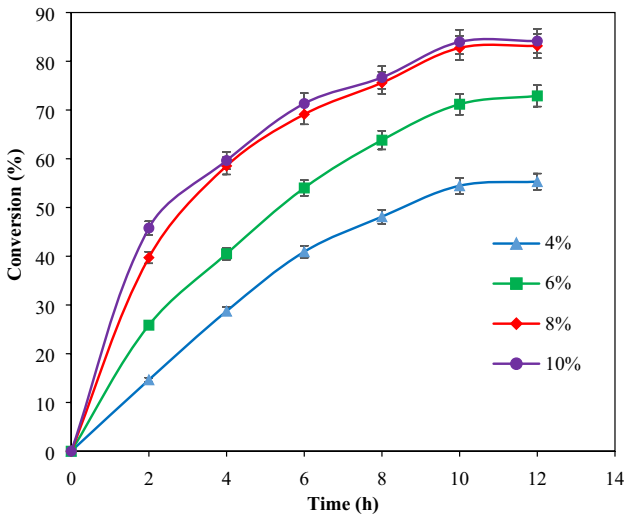

Fig. 3 Effect of biocatalyst loading on the conversion of PFAD at a temperature of 50 °C, MeOH-to-PFAD molar ratio of 1.6:1, agitation speed of 150 rpm

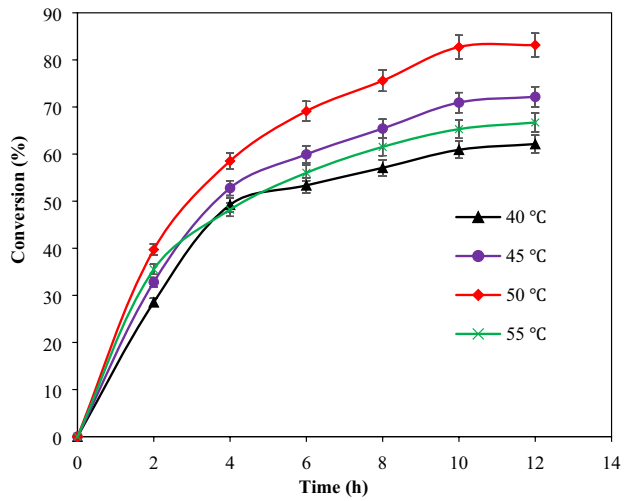

Fig. 4 Effect of temperature on the conversion of PFAD at 8 wt% biocatalyst, MeOH-to-PFAD molar ratio of 1.6:1, agitation speed of 150 rpm

3.3 Effect of temperature

Reaction temperature is an important parameter for biocatalyzed reaction, and enhances the substrate interaction by decreasing the viscosity and increasing collisions [34]. In general, the reaction rate increases with increased temperature, but because biocatalyzed reactions are temperature-sensitive, high temperature can hamper enzyme activity. Therefore, the influence of temperature on enzyme-catalyzed esterification of PFAD was examined in the range of 40–55 °C, and the results are depicted in Fig. 4. It can be seen that the percentage conversion increased with an increase in the temperature from 40 °C to 50 °C. This increase in conversion may be due to an increase in the number of collisions between the reactant and biocatalyst, thus promoting the formation of a substrate-enzyme complex and consequent increase in the conversion of PFAD.

However, when the temperature was increased to 55 °C, the conversion of PFAD decreased, owing to the denaturation of lipase molecules. From a cost perspective, the process which occurs at the lowest possible temperature and gives maximum conversion in a short time is considered the best process. Charpe and Rathod [35] studied the effect of temperature on lipase-catalyzed biodiesel production from waste frying oil and reported that the percent conversion decreased at 55 °C due to deactivation of lipase. A similar effect of temperature on esterification of palmitic acid using Novozym 435 biocatalyst was reported by Syamsul et al. [36]. In their study, maximum conversion was obtained at 40 °C, and a decrease in conversion was observed after 50 °C. Therefore, 50 °C was established as the optimum temperature for the esterification of PFAD in the present study.

3.4 Effect of molar ratio

The esterification reaction is a reversible reaction as the reaction proceeds, ester and water are formed as product and by-product, respectively; the esters are then hydrolyzed by the water into acid and alcohol. Therefore, an excess of alcohol is required to shift the equilibrium towards the product side, in addition to that the excess alcohol acts as a solvent to reduce the viscosity of the

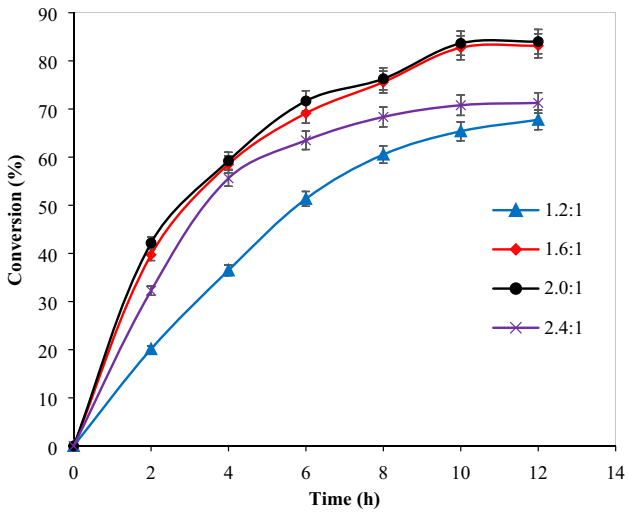

Fig. 5 Effect of molar ratio on the conversion of PFAD at 8 wt% biocatalyst loading, esterification temperature of 50 °C, and agitation speed of 150 rpm

PFAD and hence the diffusion resistance between the heterogeneous biocatalyst and reactant. On the other hand, the use of excess alcohol increases biodiesel production cost, so it is necessary to optimize the molar ratio to achieve the highest possible conversion with minimal use of alcohol.

In this regard, the effect of the molar ratio (MeOH-to-PFAD) was studied in the range of 1.2:1 to 2.4:1. Figure 5 shows that the conversion of the PFAD was increased for molar ratios of 1.2:1 (65.38%) and 1.6:1 (82.74%), while the 2:1 molar ratio showed a marginal increase in conversion. When the molar ratio was increased to 2.4:1, the conversion of PFAD decreased significantly. Yu et al. [37] reported similar results for the esterification of conjugated linoleic acid using immobilized lipase. As the molar ratio of alcohol increased from 0.5:1 to 1:1, conversion increased from 43.2% to 54.9%. However, with a further increase in the molar ratio to 2:1, conversion decreased to 38.1%. This decrease may be attributed to the inhibitory effect of alcohol (MeOH or ethanol) in higher amounts [37, 38]. Zhong et al. [39] found that short-chain alcohols, especially MeOH, are toxic to lipase, as esterification of oleic acid with MeOH yielded the lowest conversion (< 20%). Lotti et al. [40] reported that a higher concentration of short-chain alcohols might lead to enzyme unfolding and subsequent non-reversible deactivation. Thus, a MeOH-to-PFAD molar ratio of 1.6:1 was considered suitable to obtain maximum conversion by considering the cost and inhibitory action of MeOH on the biocatalyst.

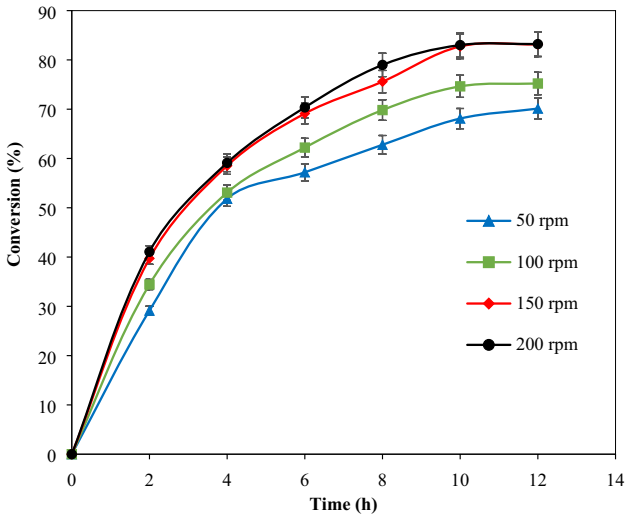

Fig. 6 Effect of agitation speed on the conversion of PFAD at biocatalyst loading of 8 wt%, esterification temperature of 50 °C, and molar ratio of 1.6:1

3.5 Effect of agitation speed

Mass transfer is a major hurdle in heterogeneous biocatalyzed reactions due to the diffusion resistance that arises between the immobilized biocatalyst and the liquid substrate. This limitation can be minimized or overcome by agitation of the heterogeneous system. However, agitation at a higher speed consumes more energy, which adds to biodiesel production costs. Therefore, the effect of agitation speed on the conversion of PFAD into FAME was examined at different rpm (50–200), and the results are depicted in Fig. 6. The figure shows that as the agitation speed increased from 50 to 150 rpm, the percentage conversion increased from 68.10% to 82.74%. The high agitation speed dispersed the immobilized enzyme (MNP-$CALB_{EX}$) homogeneously in the reaction medium, enabling the reactant molecules to more easily access the surface of the biocatalyst.

However, a further increase in the agitation speed (200 rpm) exhibited no significant increase in conversion. A similar trend was reported by Kumari et al. [41] for biodiesel production from *Jatropha* oil. In their study, enzymatic biodiesel production increased with an increase in the agitation speed from 100 to 200 rpm, but a further increase in agitation speed did not affect the conversion percentage of *Jatropha* oil. Considering the maximum PFAD conversion and cost, 150 rpm was considered as an optimal value.

3.6 Reusability of the MNP- $CALB_{EX}$

The immobilization of lipase on magnetic nanoparticles is beneficial for the recovery of the biocatalyst for utilization in successive batches. The reusability of the immobilized

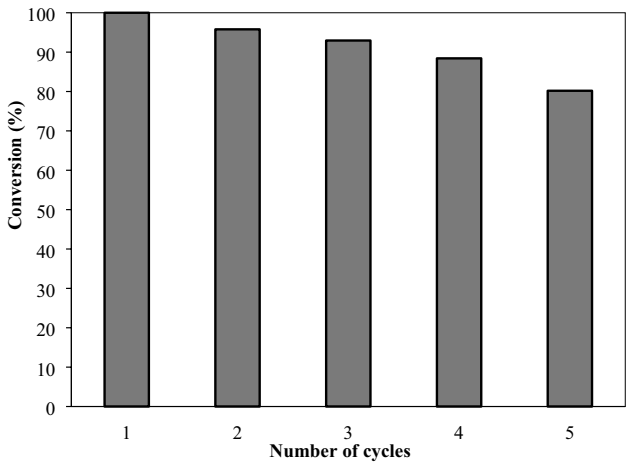

Fig. 7 Reusability of MNP- $CALB_{EX}$ biocatalyst at PFAD-to-MeOH molar ratio of 1.6:1, biocatalyst loading of 8 wt%, reaction temperature of 50 °C, and agitation speed of 150 rpm

lipase is an essential factor in the cost-effective esterification of PFAD to produce biodiesel.

The reusability of the magnetic biocatalyst was investigated at optimized operating conditions, i.e. PFAD-to-MeOH molar ratio of 1.6:1, biocatalyst loading of 8 wt%, agitation speed of 150 rpm, and temperature of 50 °C for a reaction time of 10 h. The reusability was tested up to five cycles, and the results are illustrated in Fig. 7. It can be seen that after each cycle, the residual activity of the biocatalyst was decreased. In the fifth cycle, residual conversion of 80.19% was observed. The drop in the residual conversion may be due to the denaturation of lipase after prolonged use [42].

3.7 Comparison of catalyst activity of MNP- $CALB_{EX}$ with Amberlyst-15

A comparative study was carried out to determine the efficacy of the synthesized biocatalyst with commercial Amberlyst-15 (strongly acidic ion-exchange resin). The esterification reaction was performed under (biocatalyzed) optimized reaction conditions as follows: MeOH-to-PFAD molar ratio of 1.6:1, catalyst loading of 8 wt%, reaction temperature of 50 °C, agitation speed of 150 rpm, and reaction time of 10 h. Conversion of 31.56% was obtained, which indicates that MNP-$CALB_{EX}$ was a more active catalyst in the transformation of PFAD into FAME as compared to commercially available Amberlyst-15, with MNP-$CALB_{EX}$ achieving a 2.6-fold improvement over the Amberlyst-15 catalyst. The higher conversion in the case of MNP-$CALB_{EX}$ may be due to the availability of more active sites because of the large surface area (143.26 m^2/g). Therefore, it was concluded that the current magnetic biocatalyst is more efficient than commercially available Amberlyst-15 under the above-reported reaction conditions.

4 Conclusion

In this study, immobilized lipase on Fe_3O_4 MNPs was effectively utilized to esterify PFAD to produce biodiesel. Also, 82.74% can be considered as good conversion as the used biocatalyst contain only 0.45 wt% lipase.

- MNP-$CALB_{EX}$ catalyzed esterification reaction achieved 82.74% conversion at optimized reaction conditions, i.e, molar ratio of 1.6:1, biocatalyst loading of 8 wt% (0.45 wt% lipase contain), esterification temperature of 50 °C, and agitation speed of 150 rpm.
- Under the above mentioned conditions, the commercial Amberlyst-15 catalyst provided only 31.56% conversion over a reaction time of 10 h.
- Immobilization efficiency was found to be 85%, which was provided with 56 mg of lipase/g of support.
- The immobilized biocatalyst, MNP-$CALB_{EX}$, was recycled for up to five cycles, with residual conversion of 80.19% in the fifth cycle.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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